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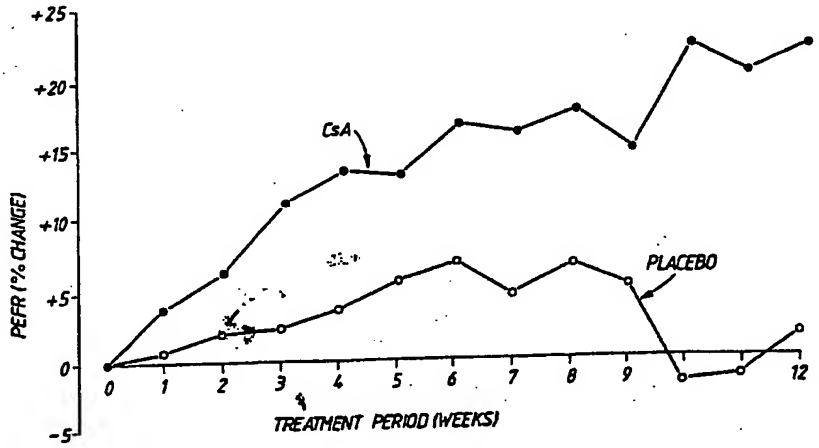

(57) Abstract

Specific pharmacological targeting of T-lymphocytes provides a new approach to the treatment of chronic asthma (both in patients relatively sensitive and resistant to the effects of corticosteroids) and to the treatment of other lung deseases such as bronchiectasis and cystic fibrosis, as well as sinusitis. The invention provides the use of cyclosporin A and other immunosuppressive agents with the same or similar mode or site of action for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis. Suitable other agents include FK 506, rapamycin and humanised anti-CD4 antibodies. The invention also provides an *in vitro* test for prediction of clinical response to corticosteroids and immunosuppressive agents. Corticosteroid resistance can be identified by the *in vitro* test and corticosteroid-resistant patients thus identified can be treated by cyclosporin A or other suitable immunosuppressive agent.

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Treatment of Lung Diseases

The present invention relates to the treatment of asthma and other diseases characterised by airflow obstruction, and to *in vitro* tests for use in connection with such treatment.

Asthma is a disease of unknown aetiology in which the bronchi (or airways) are inflamed and, as a consequence, obstructed. This narrowing results from a combination of bronchial smooth muscle contraction, mucosal oedema, inflammatory cell infiltrate and partial or total occlusion of the lumen with mucus, cells and cell debris. Bronchial obstruction is either partially or totally reversible and this important feature distinguishes asthma from chronic bronchitis (sometimes called Chronic Obstructive Airways Disease - COAD) which is caused by smoking and where there is fixed airway narrowing.

Asthma is an extremely common disease with a prevalence world-wide of between 5% and 8%. In the developed world it is the commonest chronic illness and, for reasons which are unclear, the disease is on the increase. It is now widely accepted that inflammation is an important component of the disease, but there remain many theories as to its aetiology and mechanism.

The illness has a wide clinical spectrum ranging from mild episodic bronchospasm (easily controlled by the

occasional use of a bronchodilator) to a very severe intractable asthma which is sometimes resistant to treatment with high doses of oral corticosteroids. These patients with severe chronic disease may be dependent on corticosteroids (both oral and inhaled) and their disease is often so severe that full reversibility can be difficult or impossible to demonstrate. Contrary to popular belief, moderate to severe chronic asthma, requiring either high dose inhaled and/or oral corticosteroids, is quite common, and in the U.K. accounts for many of the asthmatics followed up in hospital and out-patient clinics.

Chronic obstructive pulmonary disease (COPD) is a very common disease (particularly in the over 50 age group) and is characterised by persistent, or fixed, airway narrowing of peripheral airways, together with loss of lung recoil pressure. The terms COPD and chronic bronchitis (or chronic mucus hypersecretion) and chronic bronchitis with emphysema are sometimes used interchangeably. Emphysema occasionally occurs alone. These conditions are essentially smoking-related. COPD and asthma are generally believed to be separate disorders, but some patients may exhibit both diseases (this is then sometimes called "chronic asthmatic bronchitis"). In COPD there is bronchial hyperresponsiveness (although this is less marked than in asthma). The pathology of the bronchi in COPD is not fully elucidated, but features

include hypertrophy of mucus-secreting glands, inflammation (including infiltration with lymphocytes) and goblet cell hyperplasia.

The treatment of COPD consists of bronchodilators, intermittent courses of antibiotics and, in some patients, inhaled and/or oral corticosteroids; the latter are claimed to reduce the decline in lung function in COPD.

The diseases bronchiectasis and chronic sinusitis are final common pathways of a number of known and unknown causes of inflammation in which the first-line mucus clearance mechanism (common to both the lower respiratory bronchial airways and the upper respiratory nasal airways and para-nasal sinuses) is impaired, allowing chronic bacterial colonisation of these airways and sinuses. This colonisation stimulates chronic inflammation, resulting in progressive tissue damage, airflow obstruction and bronchial hyper-responsiveness. Treatment of bronchiectasis and sinusitis is similar to that of COPD but with more emphasis on the use of antibiotics, to modify the bacterial colonisation, and physiotherapy to eliminate infected secretions.

Cystic fibrosis is an autosomally recessively inherited condition affecting 1 in 2,500 in the U.K.. Excess viscid mucus is produced. This leads to recurrent chest infections and progressive bronchiectasis. Approximately 50% of cystic fibrosis sufferers have

bronchial hyperresponsiveness and there is an increased incidence of atopy. There is widespread airway narrowing and wheeze. Most cystic fibrosis sufferers take bronchodilators, some take inhaled corticosteroids and at least one study had reported benefit with oral corticosteroids.

Treatment of asthma can be broadly divided into (a) drugs for relief and (b) drugs for prevention. Anti-asthma drugs have a huge market world-wide which is increasing.

The most widely used drugs for relief are the selective beta$_2$-agonists, which when administered by the inhaled route usually give rapid relief of acute wheezy episodes. These drugs may be used regularly or on an "as required" basis, i.e. to relieve acute symptoms.

Apart from the mildest cases, many physicians now believe that drugs for relief should be given in combination with drugs for prevention. Drugs for prevention have to be taken on a continuous basis and "titrated" to the patient's individual requirement. There are four major groups of drugs for prevention: (1) sodium cromoglycate (Intal (Trade Mark)) or nedocromil sodium (Tilade (Trade Mark), i.e. chromone-like substances), (2) theophyllines, (3) oral "mast cell stabilisers" and (4) corticosteroids.

The chromone-like substances are effective in children but only in a minority of adults. Their great advantage is safety and the fact that they are "non-

steroid" agents. However, these preparations are generally regarded as largely ineffective in the vast majority of patients with chronic steroid-dependent asthma.

Theophyllines have been on the market since the mid-50's. They are widely prescribed and have efficacy. They are both bronchodilators and preventative. However, they can only be given by mouth and there is a narrow "window" between the toxic and therapeutic effects. Over the past few years there has been a vigorous campaign to bring to the attention of asthma doctors world-wide the potential toxic effects of theophylline.

The mast cell stabiliser drugs include ketotifen (Zaditen (Trade Mark)) and 3,4-dimethoxycinnamoyl-anthranilic acid (Tranilast (Trade Mark)). They have the great advantage of being administered by the oral route. They may have some efficacy in mild asthma in children but have to be taken for at least four to six weeks to show effect.

Corticosteroids are the mainstay of treatment of chronic asthma and they revolutionised the treatment of this disease when they were fist introduced in the 1950's. Oral corticosteroids have today been largely replaced by inhaled corticosteroids (first marketed in the late 1960's), although severe asthmatics still require medication by mouth. Inhaled corticosteroids are relatively safe and extremely effective in most patients,

although at high doses side-effects include occasional oral pharyngeal candidiasis and aphonia. The corticosteroids have improved the quality of life for millions of asthmatic sufferers world-wide.

For those with severe asthma, however, oral therapy with corticosteroids is required. When taken for more than a few days oral corticosteroids have a number of serious side-effects. These include growth retardation in children, severe osteoporosis (especially in old age), reduced responsiveness of the pituitary adrenal axis to stress, fluid retention, diabetes and precipitation of psychosis.

Furthermore, an appreciable number of asthmatic subjects have apparent corticosteroid resistance or unresponsiveness. It must also be borne in mind that patients considered to be treated successfully with inhaled or oral corticosteroids often have to be content with lung function as low as 60% of their predicted value, since attempts to improve this will often result in the necessity for increased oral corticosteroids and so run the risk of concomitant side-effects.

Thus although corticosteroids are the single most effective drug for prevention in chronic asthma, they are by no means the ideal drug, since many asthmatics are dependent on a combination of high-dose inhaled and oral steroids; even with this regime many patients have a poor quality of life and poor lung function.

Corticosteroids are both anti-inflammatory and immunosuppressive. Over the years asthma doctors have occasionally used immunosuppressive agents (which can also be called cytotoxic agents if they are used to treat cancer) as adjuncts to corticosteroids in patients with extremely severe disease, i.e. those requiring very large doses of oral prednisolone, or equivalent, for long periods of time. (The term "immunosuppressive agent" as used herein does not include corticosteroids.) One drug, azathioprine, has been used since the 60's. Clinical studies of azathioprine in asthma have shown the drug to be of limited or little benefit. Like other mercapto-purine derivatives, bone marrow depression is a serious side-effect. About four years ago considerable interest was expressed in the use of the anti-inflammatory and immunosuppressant drug methotrexate in chronic asthma since this was shown to reduce steroid requirements in chronic asthmatics. However, there is no evidence that methotrexate improves lung function, and its major side-effect, liver toxicity, probably precludes its widespread use. Increasing numbers of other side-effects with methotrexate are now being reported, i.e. opportunist infections and bone marrow suppression.

We have now found from analysis of the results of a double-blind study that the immunosuppressant drug cyclosporin A may be used safely in the treatment of severe asthma in steroid-dependent patients/those poorly

controlled by steroids. Surprisingly, the drug produced dramatic improvements in lung function in patients with severe asthma after treatment for as little as 2 to 3 weeks at a dose 1/2 to 1/3 of that employed in organ transplantation. At such doses the drug was surprisingly effective and there was no evidence of the major side-effects associated with the use of the drug.

Cyclosporin A has been in widespread use for immunosuppression in organ transplantation since the early 1980's. This compound, a natural product of a fungus, is a lipophilic cyclic peptide composed of 11 amino acids. The drug has been remarkably successful in increasing the survival of organ grafts. Doses greater than 7.5 mg/kg/day, however, are likely to be associated with reversible renal toxicity and hypertension, and this has been a particular problem in, for example, kidney transplantation. Our results suggest that cyclosporin A is effective in asthmatic patients at substantially lower doses and that, provided patients are carefully selected, the risk/benefit ratio for cyclosporin A may be no worse than (and quite possibly better than) oral prednisolone; furthermore, patients previously insufficiently controlled by existing therapy have shown an unexpectedly dramatic response.

Our clinical trial of cyclosporin A was carried out in documented steroid-dependent chronic asthmatics who were poorly controlled by even oral corticosteroid

therapy. Subjects were life-long non-smokers, demonstrated reversibility to beta$_2$-agonists, required maintenance prednisolone of 5 mg or more per day and had an FEV_1 (forced expired volume) of less than 70% predicted. All were taking high dose inhaled steroids, regular beta$_2$-agonists and theophyllines if tolerated. We excluded those with high blood pressure or with kidney problems or who were pregnant or who had a history of malignancies or chronic viral infections.

In an interim analysis carried out in respect of twenty patients who completed one limb of the study, eleven patients who had received the drug orally for 12 weeks at a dose of 5mg/kg/day were compared with nine patients who had received placebo for the same period of time. The study was totally double-blind and all patients had a one month "run-in". All other treatment, except inhaled beta$_2$-agonists, was kept constant. Twice-daily peak expiratory flow (PEFR) measurements, in addition to symptom scores and "rescue" bronchodilator aerosol usage, were recorded.

The nine placebo-treated patients showed virtually no change in PEFR over the 16 week study period. In contrast, six of the eleven cyclosporin-treated individuals had a dramatic increase in their PEFR. The effect showed after about four weeks' treatment and appeared to reach a plateau after about 8 weeks of treatment, improvements as high as 60% and even up to 182%

increases in PEFR being observed. A total of seven patients taking cyclosporin showed an improvement in PEFR (mean increase 69%, range 10-182%) and four were unchanged. Overall (even with these small numbers), the pooled results showed a 33% increase in the morning PEFR (baseline 249 ± 36, end of study 331 ± 42 l/min), and a 19% increase in the evening PEFR in those taking the active preparations. Morning PEFR was unchanged in the placebo group (baseline 235 ± 32, end of study 232 ± 31 l/min). These results were statistically significant (calculated by analysis of variance and by comparing the total response over the 12 week treatment period ["area under the curve"]).

The study has now been completed, and analysis of results for all patients shows these are extremely impressive. The frequency of disease exacerbations requiring an increase in prednisolone dose was reduced by 48% in patients on cyclosporin compared to placebo ($p<0.02$). Substantial improvements in lung function were observed in asthmatics in whom other therapies were of limited effectiveness.

Moreover, even the best asthma trials, i.e. with inhaled corticosteroids, may only show a 10% decrease in airflow obstruction. We have shown more than a 30% decrease, even with small numbers.

Surprisingly, also, the commonly-held belief that chronic severe asthma has a fixed irreversible component

might now have to be modified since we have shown, in some patients, that treatment with cyclosporin A restores the PEFR almost to predicted normal values in some of the individuals.

The drug was well-tolerated for the 12 week treatment period; thus it has been found, at least so far, to be safe at effective dosages. Changes in renal function and blood pressure could be readily controlled by monitoring the blood levels of cyclosporin A and adjusting the oral dose accordingly. Thus cyclosporin may be preferable to oral prednisolone and may prove suitable as sole therapy for severe asthma. The drug could furthermore be considered as an addition to, or alternative to, treatment even in milder disease (i.e. those taking inhaled steroids); even lower doses may be appropriate in such cases.

We can argue that cyclosporin A is an anti-inflammatory agent (as well as an immunosuppressant) since it is successful in the treatment of a chronic inflammatory disease.

We believe that treatment of other diseases characterised by airflow obstruction, more particularly COPD, bronchiectasis and cystic fibrosis will also be possible. Sinusitis might also benefit from this form of treatment.

Accordingly, the present invention provides the use of cyclosporin A or other immunosuppressant (i.e. non-corticosteroidal immunosuppressant) with the same or a

similar mode of action, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention also provides a method for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises administering a therapeutically effective dose of cyclosporin A or other immunosuppressant with the same or a similar mode of action.

The existence of such diseases may, for example, be tested by measurement of the peak expiratory flow rate or forced expiratory volume in one second (FEV_1). Thus, for example, a disease characterised by airflow obstruction exists when a test of airway obstruction such as FEV_1 gives a value significantly less than that predicted for that person's age, height, sex and racial characteristics. Alternatively/in addition, clinical history is considered; diseases characterised by airflow obstruction are, in general, characterised by shortness of breath, wheeze, an obstructive pattern of lung function or chest tightness or by two or more such features.

Whereas at one time mast cells and their products were believed to account for most of the symptoms and pathological changes of asthma, we now believe that the T-lymphocyte plays a central role. Preliminary work by us has shown that even in intrinsic asthma there are activated CD4+ T cells in mucosal bronchial biopsies;

thus asthma can, we believe, be regarded as an immunological disorder with many features in common with autoimmune disease.

We have carried out an immunohistological study of the bronchial mucosa in occupational, intrinsic and extrinsic asthma. The results support the hypothesis that T-lymphocyte/eosinophil interactions are important in the pathogenesis of asthma of diverse aetiology.

We believe that asthma is associated with the elaboration of cytokines from activated CD4+, IL-2R+, T helper lymphocytes located in and around the bronchial mucosal surface. These CD4+ lymphocytes may be a specialised subset of T cells which orchestrate the intense inflammatory reaction around the bronchi. We believe the activation of the CD4+ cells to be the underlying cause of bronchial hyperresponsiveness, a cardinal feature of the disease.

We believe also that activated T cells are probably the main target for corticosteroids in asthma and that corticosteroid resistance might be associated with activated T cells which do not respond to such drugs; in unresponsive patients the steroids are, we believe, unable to suppress activated T CD4+ cells/macrophages.

Unlike the immunosuppressants such as azathioprine and methatrexate, cyclosporin is known to have a much more specific mode of action, i.e. it suppresses the production of interleukin 2 (IL-2) by T lymphocytes (and

thereby inhibits the elaboration of the essential T cell growth factor). We believe the CD4+ T lymphocytes are involved. Although cyclosporin has been shown to have other modes of action on, for example, macrophages (and other antigen-presenting cells), basophils and mast cells, CD4 T lymphocytes appear to be its principal target.

As regards COPD, even asymptomatic smokers have lymphocyte infiltration into the bronchial mucosa, indicating that T cell-mediated damage may be an early event in the development of COPD. Also, there is evidence from animal studies to suggest that goblet cell hyperplasia may be under T-lymphocyte control. Accordingly, we believe that immunosuppressants such as cyclosporin A also have potential in the treatment of COPD.

As regards bronchietasis, the chronic inflammatory response in this disease consists of a bronchial intramural cell mediated immune response and traffic of neutrophil polymorphonuclear leucocytes through the bronchial wall to the bronchial lumen. We have found that the cell-mediated immune response consists of increased numbers (and activation) of T-lymphocytes of CD4 and CD8 lineage (the latter being present in large numbers in all compartments of the bronchial wall, the former present in follicles) and of antigen processing and mature macrophages.

Accordingly, we believe that immunosuppressants such as cyclosporin A also have potential in the treatment of bronchiectasis and sinusitis, and trial results to date bear this out.

Furthermore, we have found that the mean number of T lymphocytes (recognised by the monoclonal antibody MT1) in asthma and cystic fibrosis groups was approximately twice that of a control group ($p<0.05$ and 0.01 respectively). Accordingly, we propose the use of cyclosporin A or other immunosuppressant for the treatment of cystic fibrosis.

Thus, the present invention also provides the use of cyclosporin or other immunosuppressive agent which prevents the release of cytokines from T cells, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention also provides the use of a T cell-selective immunosuppressive agent for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention also provides the use of an immunosuppressive agent that inhibits the elaboration of cytokines such as IL-2, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention further especially provides a method for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises administering a therapeutically effective dose of cyclosporin or other immunosuppressive agent which prevents the release of cytokines from T cells.

The present invention further especially provides a method for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises administering a therapeutically effective dose of a T cell-selective immunosuppressive agent.

The present invention also provides a method for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises administering a therapeutically effective dose of an immunosuppressive agent that inhibits the elaboration of cytokines such as IL-2.

We have also investigated the cytokine mRNA profile and T-cell activation in bronchoalveolar lavage cells in atopic asthma (that is, extrinsic or allergic asthma). We found that atopic asthma is characterised by T cell activation, mRNA for cytokines of the "IL-4 family" (IL-3, IL-4, IL-5 and GM-CSF) and local eosinophilia. TNF is also released and has importance in the asthma process; IL-8 is probably also released and may have importance in the process.

Thus, we believe it likely that several cytokines

(lymphokines) are important in asthma. They include IL-2, IL-3, IL-4, IL-5, GM-CSF (granulocyte/macrophage-colony stimulating factor), TNF (Tissue Necrosis Factor), IL-6 and IL-8. In combination these are important in the asthma process, and especially critical, we believe, is IL-5 because that produces local eosinophils.

Preferably the immunosuppressive agent used according to the present invention is specifically targeted at the $CD4^+$ T helper cell.

The agent is preferably selective for the IL-4 family of cytokines, especially IL-5.

As well as cyclosporin, other suitable immunosuppressive agents include, for example, FK 506 and rapamycin. The use of anti-T cell antibody, especially anti-CD4 antibody, anti-IL-2 receptor monoclonal antibody and antibodies designed in a similar fashion to CAMPATH-1H (reshaped by genetic engineering to resemble more closely a human antibody ("humanised")) is also contemplated.

The product FK506, manufactured by the Fujisawa Company, Japan, is, like cyclosporin, a natural by-product of a soil fungus and is a potent inhibitor of interleukin-2 production. In contrast to the peptide structure of cyclosporin, FK506 is a macrolide antibiotic (like erythromycin). Although this agent has not been fully evaluated in organ transplantation, it appears to be approximately 100 times more potent than cyclosporin

in murine and human mixed lymphocyte reactions, and in recent clinical trials it has been found to be extremely effective in suppressing rejection of kidney transplants. The toxicity associated with doses of FK506 relevant for transplantation purposes is, however, only beginning to be defined.

Its mode of action is very similar to that of cyclosporin, i.e. it inhibits the early events associated with lymphocyte activation by preventing the synthesis of IL-2 and other lymphokines important in lymphocyte growth and function, and it appears that there is a single site of action for both FK506 and cyclosporin (Lancet, Vol 338: Sept 28 1991, 789). It appears that each compound binds to similar but different proteins (immunophilins), both of which are peptidyl-prolyl isomerases, enzymes that promote the folding of their substrates. It has been shown that a drug-immunophilin complex binds to calcineurin (a protein phosphatase), and it is posible that cyclosporin and FK506 might inhibit the phosphatase activity of calcineurin, thereby preventing an essential dephosphorylation step in cytosolic NF-AT (nuclear factor of activated T cells) processing, which in turn might prevent both translocation of cytosolic NF-AT to the nucleus and subsequent interleukin-2 gene transcription.

The present invention further provides the use of FK506 or other immunosuppressive agent with the same or similar principle of action (or same or similar mode

and/or site of action), for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention further provides the use of cyclosporin or FK506 or other immunosuppressive agent with a principle (or mode and/or site) of action in common with either or both, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

FK506 (also called FR-900506) is described and claimed in EP 0184162 A. That European patent application also describes and claims other compounds, their preparation and use as immunosuppressants. The present invention also includes the use of those compounds, of the general formula I described in EP 0184162 A and their salts, more especially the compounds specifically mentioned (FR-900520, FR-900523 and FR-900525), for the treatment of asthma and other diseases characterised by airflow obstruction and/or of chronic sinusitis.

Another compound, the antifungal product rapamycin (Calne R.Y. et al., Rapamycin for immunosuppression in organ allografting, Lancet 1989; 2:227), is structurally similar to FK506 and has also been found to have immunosuppressive effects.

The present invention further provides the use of cyclosporin or rapamycin or other immunosuppressive agent with a principle (or mode and/or site) of action in

common with either or both, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

Antibodies directed against the CD4 subset of T lymphocytes are, for example, VIT4 and M-T151. Such antibodies have been found to be effective in rheumatoid arthritis. Reiter C. et al (Arthritis & Rheumatism 1991 34, 525) and Walker C. et al (J. of Autoimmunity 1989, 2, 643-649) describe the use of the CD4 monoclonal antibody M-T151 and Herzog C. et al (J. of Autoimmunity 1989 2, 627-642) describe the use of M-T151 and VIT4. More recently a chimeric IgG monoclonal anti-CD4 antibody (M-T412) manufactured by Centocor, Inc. has become available, and there is also a similar murine-human chimeric antibody (chimeric 17-1A) (LoBuglio A.F., Wheeler R.H., Trang J et al. Mouse/human chimeric monoclonal antibody in man: kinetics and immune response, Proc Natl Acad Sci USA 1989, 86:4220-4).

Soulillou J.P. et al. (N. Engl. J. Med. 1990, 322:1175-82) has investigated anti-interleukin-2-receptor therapy in renal transplantation and shown that such an antibody can be beneficial in preventing rejection. Similar results are being obtained with another anti-interleukin-2-receptor antibody (Carpenter C.B. et al., Am. J. Kidney Dis., 1989; 14: Suppl. 2:54-7). The monoclonal antibody 33B3.1 (Soulillou J-P et al., N. Engl. J. Med., 1990, 322, p1175-1182), which is also an

antibody against the interleukin-2 receptor, should also be mentioned. Such monoclonal antibodies are also contemplated for use in the present invention.

The present invention further provides the use of an antibody directed against the IL-2 receptor and/or against one or more cytokines selected from the IL-5, IL-4, IL-3 and GM-CSF family of cytokines (i.e. the IL-4 family of cytokines), IL-6, TNF, IL-8 and IL-10 and/or against IFN-gamma, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis. The antibody is preferably humanised.

Another antibody effective in the reversal of rejection and directed only against T lymphocytes is anti-CD3 (Cosimi A.B. et al., N. Engl. J. Med. 1981, 305: 308-314), now licensed as Orthoclone OKT3, which is a monoclonal antibody to T cells expressing the CD3 antigen. This is less specific than the antibodies mentioned above, but may be suitable in certain cases.

US Patent 4,117,118 describes and claims cyclosporin A and cyclosporin B and their use inter alia as antibiotics and as immunosuppressants. The present invention also includes the use of cyclosporin B for treatment of asthma and other diseases characterised by airflow obstruction and/or of chronic sinusitis.

The use of the prostaglandin E_1 analogue misoprostol in conjunction with cyclosporin or other appropriate

immunosuppressive drug should also be mentioned. We believe that when used in appropriate combination therapy, for example with cyclosporin, the drug will potentiate the effect of the immunosuppressant.

As indicated by the data from our trial, "local immunosuppression" produced by the various immunosuppressive drugs mentioned above may be highly effective in the treatment of asthma, particularly chronic asthma, and other diseases characterised by airflow obstruction and/or of chronic sinusitis.

Dosages of oral cyclosporin of, for example at least 1 mg/kg/day, especially at least 5 mg/kg/day, and no more than 7.5 mg/kg/day, especially no more than 10 mg/kg/day, should be mentioned. These may, for example, be given in two divided doses. Oral dosages for other immunosuppressant drugs may be higher or lower, depending on their activity, but in general doses substantially less than those required for transplantation purposes, for example 1/3 to 1/2 of such doses, or even less for mild cases, may be appropriate. Oral doses of FK506 of from 0.075 to 0.15 mg/kg/day, given for example in two divided doses, may for example be mentioned.

The preparation of oral dosage forms may be carried out by methods known in the art. Suitable cyclosporin-containing preparations for oral administration contain the active ingredient, for example in an amount of 25 to 250 mg per dosage unit. Suitable oral dosage forms

containing FK506 contain the active ingredient, for example in an amount of 2.5 to 5 mg per dosage unit. Pharmaceutical preparations suitable for immunosuppressant use containing FK506 are described in EP 0184162 A; as mentioned above, for the present invention dosages will generally be 1/3 to 1/2 of such dosages. Pharmaceutical preparations containing FK506 as a solid dispersion to achieve solubilisation of the drug and improve bioavailability are described in EP 0240773 A.

It is clear that safety may be maximised by delivering the drugs by the inhaled route either in nebuliser form or as a dry powder. Clearly the great advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or of chronic sinusitis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

Preparation of forms suitable for administration by inhalation may be carried out by methods known in the art. It should be noted that several antibiotics have recently been developed for topical inhaled usage, particularly in cystic fibrosis, where they have been shown to be effective against pseudomonas infections. Various inhalants are described, for example, in DE 1491707, GB 1,392,945, GB 1,457,351, GB 1,457,352, NL 147939, DE 1491715, GB 1,598,053, EP 5585, EP 41783, EP 45419, EP 360463 and FR 2628638. DE 1491715, in

particular, is said to be suitable for inhalation therapy intended for bronchial or lung diseases.

Accordingly, the present invention provides the use of cyclosporin A or other immunosuppressant with the same or a similar mode and/or site of action, for example FK506 or rapamycin or other immunosuppressive agent with a common principle of action, especially a T cell-selective immunosuppressive agent or an immunosuppressive agent that inhibits cytokine, e.g. IL-2, production, for the manufacture of a topical preparation for the treatment, with or without concurrent use of other drugs, of diseases characterised by airflow obstruction and/or of chronic sinusitis.

The present invention further provides a pharmaceutical preparation for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises cyclosporin A or other immunosuppressant with the same or a similar mode and/or site of action, for example FK506 or rapamycin or other immunosupprssive agent with a common principle of action,especially a T cell-selective immunosuppressive agent or an immunosuppressive agent that inhibits cytokine, e.g.IL-2 production, if desired together with a further agent, for example misoprostol, in admixture or conjunction with a pharmaceutically suitable carrier, and which is in a form suitable for inhalation.

As mentioned above, the immunosuppressive agent may

be selective for the IL-4 family of cytokines, for example IL-5.

Dosages for the topical preparation will in general be one tenth to one hundredth, for example one twenty-fifth, of the dose required for oral preparations.

We have also shown that patients with corticosteroid-dependent asthma can be divided into corticosteroid responders and corticosteroid-resistant subjects. The clinical response to oral prednisolone (a type of corticosteroid commonly used) is reflected by the behaviour of the T-lymphocytes in vitro. When T-lymphocytes are incubated with a lectin (phytohaemagglutinin - PHA) they proliferate and incorporate tritiated thymidine. In normal subjects and those patients who are corticosteroid-responsive, this proliferation is inhibited by dexamethasone (another corticosteroid) at low concentrations (10^{-7} to 10^{-9} mol/l). In contrast, the T-lymphocytes from corticosteroid-resistant asthmatics still proliferate in the presence of dexamethasone (at these same concentrations), indicating that the clinical response and the in vitro response of the T-lymphocytes occur in parallel. Thus we have found that corticosteroid inhibition of T cell proliferation can be used as a predictive test for the response of patients to corticosteroids. We propose that cyclosporin A can be used in the same way, as can other immunosuppressants, including rapamycin, CAMPATH-H, FK-506 and analogues.

Thus the response of T cells from chronic asthmatics to PHA-induced proliferation in the presence of these agents would predict clinical response, thus obviating the necessity for a formal clinical trial of the drug. Also, it may be possible to assess the most suitable corticosteroid or non-corticosteroid immunosuppressant for an individual patient by a prior screen of their lymphocytes in the presence of the various drugs. In support of this is the observation that some patients who are corticosteroid-resistant and whose cells proliferate in the presence of dexamethasone nevertheless show inhibition in the presence of cyclosporin (indicating that they may clinically respond to cyclosporin A). We found that, of four patients having T-lymphocytes resistant in vitro to corticosteroids, two responded dramatically to cyclosporin A therapy.

The proposed tests include not only inhibition of proliferation by PHA (and other lymphocyte mitogens) but also measurements of the degree of inhibition of the cytokines they release, i.e. IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, TNF, GM-CSF or Interferon-gamma. Thus cytokine production after stimulation (as opposed to tritiated thymidine incorporation) can be used as the end-point.

Variants of the above-described tests include inhibition of proliferation of lymphocytes stimulated by immobilised anti-CD3 and stimulation with common antigens to which most of the population are sensitive (i.e.,

purified protein derivative from M tuberculosis, Candida and mumps antigens) and also stimulation of T lymphocyte blasts with interleukin-2.

Accordingly, the present invention provides a method of predicting clinical response to a corticosteroid or an immunosuppressive agent in a treatment for asthma or other disease characterised by airflow obstruction and/or of chronic sinusitis, which comprises ascertaining the effect of a corticosteroid or immunosuppressive agent on the response of T-lymphocytes or T-lymphocyte blasts *in vitro* to treatment with a stimulant or mitogen, an inhibitory effect by the corticosteroid or immunosuppressive agent on the response of the T-lymphocytes or T-lymphocyte blasts to the stimulant or mitogen being indicative of a positive response *in vivo* to therapy by one such compound and/or an absence of such inhibitory effect being indicative of a negative response *in vivo* to therapy by one such compound.

Suitable immunosuppressive agents for the test are cyclosporin A and other immunosuppressive agents with a common principle (or mode and/or site) of action; they may be immunosuppressive agents that inhibit cytokine elaboration. T cell-selective immunosuppressive agents and immunosuppressive agents that are selective for the IL-4 family of cytokines should especially be mentioned. Thus, suitable immunosuppressive agents may be, for example, humanised antibodies directed against the IL-2

receptor and/or against one or more of IL-5, IL-4, IL-3 and GM-CSF. Suitable corticosteroids are, for example, prednisolone and dexamethasone. The use of dexamethasone, prednisolone, cyclosporin A, rapamycin, CAMPATH-H, VIT4, M-T151, M-T412 or FK-506 or an analogue thereof or of misoprostol together with cyclosporin or one of the other agents should especially be mentioned. The compound or mixture of compounds used in vitro in the T-lymphocyte test may, for example, be the same as that (those) for which prediction of response to therapy is sought.

The in vitro test may be used to establish the prima facie suitability or lack of suitability of a particular patient for treatment by a corticosteroid or immunosuppressive agent. A patient who is unresponsive to one particular therapy may be responsive to treatment with a different agent; for example a corticosteroid non-responder may be shown to be likely to respond to treatment by an immunosuppressive agent. Thus, preferably, clinical treatment is carried out with a compound used in the test that gives the inhibitory effect in vitro or with a related compound or one known to have similar action; if more than one compound is tested, the compound that gives the greatest inhibition or a related compound or compound having similar action may be selected. When quantitative measurements are taken the test may be used to give an indication of dose for

clinical treatment. In such cases the in vitro test may be used to establish the most effective therapy for a particular subject.

Alternatively, or in addition, the test may be used to screen potential immunosuppressive agents, for example by carrying out the in vitro test on T-lymphocytes from a normal subject or proven immunosuppressant responder and/or by carrying out tests on T-lymphocytes from a number of subjects.

In tests with PHA we found that T lymphycytes from patients with steroid-resistant asthma responded in the same way to cyclosporin A as T-lymphocytes from patients with steroid-sensitive asthma (n = 6 and 5 respectively). This and other data suggests that corticosteroid-resistant asthmatics clinically respond to cyclosporin A. Thus, not only can corticosteroid-resistance be identified by an in vitro test (T cell proliferation), but it appears that corticosteroid-resistant patients thus identified will respond clinically to cyclosporin A.

The following Examples illustrate the invention.

EXAMPLE 1

CYCLOSPORIN A IN CORTICOSTEROID-DEPENDENT CHRONIC SEVERE ASTHMA

Patients

Patients were aged 18-65 and were required to have forced expiratory volume in one second (FEV_1) and/or peak expiratory flow (PEFR) of less than 75% of the predicted value during the run-in period despite requiring long-term maintenance prednisolone of 5-20mg/day in addition to maximal tolerated and effective additional therapy. An attempt was made to establish the optimum dose of oral prednisolone for each patient prior to the run-in period. All subjects had to demonstrate greater than 20% reversibility in PEFR or FEV_1 after inhaled salbutamol during the run-in period. Entry criteria were designed to exclude subjects with smoking-related airflow obstruction and other pulmonary disease, as well as patients with contraindications to cyclosporin A (CsA) therapy.

Study design

After a four week run-in period subjects were randomised, using a randomisation table, to receive CsA (initial dose 5mg/kg/day) or matching placebo for 12 weeks. Following a two week washout period subjects crossed over to placebo or CsA medication for a further 12 weeks. The trial ended with an eight week run-out period without trial medication. Asthma severity and adverse events were monitored throughout using both

subjective and objective data collected by a blinded trial physician. Throughout the study patients had 24 hour access to the trial physician via an aircall bleep.

The primary outcome measures were variations in mean morning and evening PEFR and the number of disease exacerbations requiring a temporary increase in prednisolone dose. Secondary outcome measures were alterations in FEV_1, forced vital capacity (FVC) and slow VC measured at clinic visits, and diurnal variation in PEFR, subjective symptom scores and inhaled bronchodilator consumption as recorded on asthma diary cards.

The trial was designed to detect an improvement in lung function rather than a corticosteroid-sparing effect. The crossover design allows direct intra-subject comparisons with each patient acting as his/her own control. For this reason all treatment except inhaled bronchodilator usage was kept constant during the trial, although prednisolone dosage was increased according to standard clinical practice where necessary for exacerbations. An exacerbation was defined as an increase in asthma symptoms and/or a decrease in PEFR, taking into account individual features. Exacerbations were treated along standard clinical lines tailored to each patient according to previous experience. Patients who had exacerbations requiring an increase in prednisolone above 40mg at any time, 40mg daily for more than two weeks or hospital admission were withdrawn from study.

Clinical protocol

Screening day. Compliance with entry criteria was established by clinical history and examination and the following baseline investigations: urinalysis, microscopy and culture; full blood count and differential count; serum biochemistry (creatinine, urea, electrolytes, bilirubin, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), calcium, phosphate, urate); serum protein electrophoresis; hepatitis B serology; total serum immunoglobulin (Ig) E; chest radiograph; FEV_1, FVC and slow VC pre- and post-salbutamol; carbon monoxide gas transfer.

Run-in period. The screening day was followed by a four week run-in period during which baseline PEFR, symptoms and bronchodilator usage were recorded. Patients completed an asthma diary card twice daily, recording PEFR using a mini-Wright peak flow meter before and 15-30 minutes after β_2-agonist (3 attempts, all values recorded), inhaled bronchodilator usage (number of puffs and number of nebulisers) and a symptom score (0-3 for the previous day or night: 0=no symptoms, 1=mild, 2=moderate, 3=severe).

Entry day. Patients who completed the run-in period and in whom the results of the screening investigations were satisfactory attended for repeat spirometry pre- and post-salbutamol, repeat haematology and biochemistry,

skin prick tests to common aeroallergens and pregnancy test in premenopausal women. Isotope glomerular filtration rate (GFR) was measured and, if normal, the patient was enrolled into the study. Written informed consent was obtained from each patient and the study was approved by the ethics committee of the Royal Brompton National Heart and Lung Hospitals.

Treatment periods. Patients attended the clinic weekly for the first four weeks of each treatment period and thereafter every fortnight. Cyclosporin A in oily suspension or identical placebo was initially given at a dose of 5mg/kg/day in two divided doses. At each clinic visit asthma diaries were examined and spirometry performed; asthma symptoms, possible adverse effects, changes in concomitant medication, blood pressure and volume of trial medication used since the last visit were recorded; haematology and biochemistry (as on the screening day) and urinalysis were performed; and whole blood trough concentrations of CsA were measured at the Analytical Unit, Dept. of Cardiological Sciences, St. George's Hospital Medical School, using the specific monoclonal Cyclo-Trac SP radioimmunoassay (Incstar Corp., Stillwater MN). At the end of each treatment period isotope GFR, serum protein electrophoresis and pregnancy test (in premenopausal women) were repeated. Patients were monitored as in the rest of the trial during the eight week run-out period with clinic visits at weeks

four and eight.

Cyclosporin A dosage. Cyclosporin A concentrations and renal function were monitored by an open investigator, who took no part in patient management during the trial. The blinded trial physician received instructions prior to patients' visits to increase or decrease the dose of trial medication by a certain percentage or to leave the dose unchanged. To maintain blindness, changes were also made when patients were taking placebo. Whole blood trough concentrations of CsA between 100-250µg/l were deemed satisfactory and dosage was adjusted to achieve levels within this range. In addition, the dose of CsA was decreased if serum creatinine rose above 130% of baseline or above the upper limit of the normal range, if serum potassium rose above the upper limit of the normal range or if hypertension developed.

Isotope GFR. Twenty-three of the 33 patients had GFR measured at The London Chest Hospital by a modification of the ^{99m}Tc-(Sn)DTPA arm counting method of Macleod et al.. The remaining 10 patients attended The Royal Marsden Hospital, Kensington, where GFR was measured using the ^{51}Cr technique with venous sampling at three hours. Percentage change in GFR was used for analysis since the two techniques have different normal ranges.

Statistical analysis

This was performed by an independent medical statistician. Data collected daily (diary card) was

summarised as a mean for the last four weeks of each treatment period and used in an intention-to-treat analysis of within-patient differences between active and placebo treatments. This takes into account the intrinsic variability of airflow obstruction in asthma and the possible delay in onset and carryover of any therapeutic effect. The data used was from weeks 9-12 for patients completing a treatment period and from the last four weeks of treatment for patients who failed to complete a treatment period. Data collected at clinic visits was also analysed on an intention-to-treat basis for within-patient between treatment differences using data from week 12 or, where patients failed to complete a treatment period, from the last clinic visit.

The within-patient differences between treatments (active minus placebo) for each of the diary and visit variables were summarised as above. The treatment effect was calculated combining a paired Student's t test on each group as recommended by Pocock for crossover data. The period and differential carryover effects were calculated following the same method. (The duration of sustained drug effect on morning PEFR after cessation of CsA therapy was calculated using a simple paired t test of the diferences between the mean run-in value and each week from the end of CsA treatment.) The number of exacerbations requiring an increase in prednisolone dose was summarised for each patient (placebo minus active)

and analysed by the Wilcoxon matched pairs signed rank test. A two-tailed probability of 5% or less was taken as being statistically significant.

Results

Thirty-three patients were enrolled into the trial. Their baseline (pre-trial) demographic characteristics, medication and lung function are shown in Table 1. All patients had long-standing corticosteroid-dependent asthma, with a mean FEV_1 of 60.1% of the predicted value despite therapy with oral prednisolone in addition to high doses of inhaled corticosteroids, inhaled bronchodilators and, where tolerated, oral theophylline. Two patients had repeated hospital admissions for asthma in the washout period, and the trial had ended before they were able to cross over to the second treatment period, one to CsA and one to placebo. (The latter had been withdrawn during week 9 CsA treatment because of an acute exacerbation requiring hospital admission.) One patient withdrew after the end of the first (CsA) treatment period because of hypertrichosis. Thirty patients therefore received both CsA and placebo medication, and data from these patients have been used in the intention-to-treat crossover analysis. Fifteen received CsA in the first treatment period and fifteen placebo.

Twenty-six of these thirty patients fully completed the protocol. Three patients were withdrawn during placebo treatment, two (weeks 7 and 10, second

period) because of acute exacerbations requiring hospital admission, and the third (week 7, second period) because of an exacerbation requiring an increase in prednisolone dose above 40mg daily. A fourth patient was withdrawn from CsA treatment (week 10, second period) for investigation of facial swelling, subsequently attributed to the introduction of daily oral prednisolone seven months prior to the start of the study.

Cyclosporin A therapy resulted in significant increases above placebo in both morning and evening PEFR both pre- and post-bronchodilator (Table 2). These increases did not appear to have reached a plateau after 12 weeks of treatment. Fig. 1 shows the percentage change from baseline in mean weekly morning PEFR for the CsA and placebo treatment periods. Percentage change is shown since the mean baseline for the placebo period (266.9 l/min) was higher than that for CsA (247.6 l/min) because of the improvement in mean PEFR in those patients who received CsA in the first treatment period. There was marked variation in the PEFR response between individuals: six patients improved by over 25% above placebo, two of these by more than 50%, whereas others showed little or no improvement. The percentage increase in PEFR after inhaled bronchodilator was only slightly reduced by CsA treatment, the improvement in PEFR with CsA being in addition to, rather than in place of, the β_2-agonist-sensitive component. The sustained treatment

effect of CsA on morning PEFR following cessation of CsA therapy was calculated using data from the run-out period in patients who received CsA second and from the washout and placebo periods in patients who received CsA first. Analysis using a simple paired t test revealed a significant increase in mean morning pre-bronchodilator PEFR above baseline (run-in) values of 33-58ml (12.8-22.5%) for each of the first eleven weeks after cessation of CsA therapy.

Patients on CsA suffered significantly fewer exacerbations requiring rescue prednisolone compared to placebo, thirteen having more exacerbations on placebo than on CsA and four having more on CsA than placebo ($p=0.023$). There was a reduction of 48% in the frequency of exacerbations on CsA therapy as compared to placebo (0.84 vs. 0.44 exacerbations per patient per 12 week treatment period $p<0.02$).

FEV_1, FVC and slow VC increased significantly above placebo with CsA treatment (Table 2). As with PEFR, the increases in FEV_1 and FVC (but not slow VC) did not appear to have reached a plateau by the end of the 12 week period. The variability in individual response was large, with a range of -10% to 100%.

Cyclosporin A therapy produced a larger mean improvement in morning PEFR as compared to evening PEFR, with a corresponding reduction in diurnal variation in PEFR from a mean of 31.2 ml on placebo to 21.6 ml on CsA

(p=0.04). There was a trend (p=0.08) towards a reduction in mean daily symptom score on CsA compared to placebo but no significant difference in bronchodilator usage. Significantly more patients reported at the end of the trial that their asthma was subjectively better during CsA treatment compared to placebo (21 vs. 8, p=0.02), with one patient reporting no difference between the two treatments.

No statistically significant differential carryover or period effects were observed in the within-patient between treatment crossover analysis of any of the outcome measures. However, although not statistically significant, such effects may nevertheless hold clinical importance, as observed with the sustained treatment effect on morning PEFR of 11 weeks after cessation of CsA.

There were statistically significant increases in systolic and diastolic blood pressure, total white cell count (without significant changes in individual elements), serum potassium, urea, globulin and ALP with CsA therapy compared to placebo (Table 3). One patient required diltiazem after eight weeks of CsA treatment but was normotensive without diltiazem by the end of the trial. Although there was a significant rise in mean serum creatinine of 8.8μmol/l (p<0.001) above the baseline (run-in) value during CsA therapy, the increase above placebo did not reach significance (p=0.2). There

was a trend (p=0.06) towards an increase (of 27μmol/l) in serum urate. There was no significant change in blood eosinophil count from a mean baseline of $0.25x10^9$/l. All blood results which became abnormal during CsA therapy returned to baseline values by the first clinic visit after the CsA treatment period. The mean (SE) CsA dose for the last four weeks was 5.22 (0.33)mg/kg/day (range 2.3-8.8) with whole blood concentrations of 151.7 (7.2) μg/l (range 46-217) taken 17 (0.45) hours post dose. There was no correlation between CsA dose and CsA blood concentrations.

Cyclosporin A therapy caused a significant mean (SE) decrease in GFR of 5.7 (2.6)% compared to placebo (p<0.02). There was no difference between baseline GFR values and measurements at the end of the second (placebo) treatment period in patients who received CsA first, suggesting that the decrease in GFR observed with CsA was reversible. Symptoms reported by patients are shown in Table 4. Apart from hypertrichosis, which took up to four months to resolve completely, all adverse symptoms attributed to cyclosporin resolved within four weeks.

Discussion

The improvements in lung function with CsA therapy appeared to be continuing to increase at the end of the 12 week treatment period and it is possible that further

improvements might have been observed if treatment had been continued for longer.

The length of the carryover effect varied greatly between individuals. Mean PEFR measurements in one of the best responders (a woman with toluene diisocyanate-induced asthma) returned to run-in values within two weeks of cessation of CsA. In contrast, another individual who received CsA in the first treatment period maintained her improvement in PEFR almost undiminished to the end of the runout period, resulting in a large increase in PEFR above run-in values but only a modest increase compared to placebo. The presence of this carryover effect in patients who received CsA in the first treatment period acts to decrease the observed improvement compared to placebo. Similarly, the larger number of rescue courses of prednisolone required by the patients on placebo therapy acts to increase PEFR more than in the CsA group, thus further decreasing the observed effect of CsA on lung function as compared to placebo. Although improvements in lung function with CsA therapy reverted rapidly in some patients on cessation of treatment, there was no evidence of rebound exacerbations with PEFR levels below run-in values after cessation of CsA.

Four patients achieved improvements in pulmonary function which were greater than those recorded since becoming oral corticosteroid-dependent, even during

periods of high doses of prednisolone. This improvement was in airflow obstruction which would previously have been considered to be "irreversible".

TABLE 1 - BASELINE (PRE-TRIAL) PATIENT CHARACTERISTICS, TREATMENT AND RESPIRATORY FUNCTION DATA

Males:Females (n)	13:20
*Age	49 (31-64)
*Duration of asthma in years	27 (4-54)
*IgE (IU/ml)	146 (6-1250)
Skin prick test +ve (n)	20
*Continuous oral corticosteroids (years)	9.3 (0.3-25)
*Prednisolone dosage (mg/day)	8.5 (5-20)
+Inhaled corticosteroid usage (µg/day)	1665 (91)
Oral theophylline usage (n)	23
+PEFR [%predicted]	239 (19.3) [54.1%]
+FEV_1 [%predicted]	1.73 (0.14) [60.1%]
+VC [%predicted]	3.09 (0.21) [82.8%]

*mean (range)

+mean (standard error of the mean)

TABLE 2 - LUNG FUNCTION RESULTS*

	%diff	95% CI		p
Morning PEFR				
pre-bronchodilator	12.0	4.61,	19.39	0.0037
post-bronchodilator	10.3	2.8,	17.7	0.009
Evening PEFR				
pre-bronchodilator	6.5	0.52,	12.44	0.024
post-bronchodilator	5.5	-0.35,	11.39	0.038
FEV_1	17.6	7.34,	27.84	<0.001
FVC	10.5	4.88,	16.08	<0.001
Slow VC	8.1	2.89,	13.21	0.006
	diff	95% CI		p
Diurnal variation (ml)+	-8.6	-16.3,	-0.88	0.041
Symptom score	-0.14	-0.3,	0.02	0.08^
Bronchodilator usage (puffs)	-0.27	-0.94,	0.4	0.40^

* Intention to treat analysis of the last 4 weeks of treatment for PEFR and diary card data and of the last visit for spirometry data.
Abbreviations: %diff, percentage difference between CsA and placebo; diff, absolute difference; 95% CI, 95% confidence intervals for the %diff or diff; p, probability.

+ mean evening PEFR minus mean morning PEFR

^ not statistically significant

TABLE 3 - SAFETY DATA*

	CsA	Placebo	Change (SE)	p
Systolic b.p. (mm Hg)	138.0	128.2	9.6 (2.50)	<0.001
Diastolic b.p. (mm Hg)	86.5	78.4	7.3 (2.17)	0.003
Total wbc ($x10^9/l$)	10.6	9.68	0.91 (0.41)	0.03
Neutrophils ($x10^9/l$)	7.9	6.95	0.87 (0.44)	0.06+
Potassium (mmol/l)	4.2	3.9	0.27 (0.09)	0.005
Urea (mmol/l)	6.7	5.2	1.54 (0.36)	<0.001
Creatinine (μmol/l)	94.2	91.6	3.5 (2.64)	0.20^+
Globulin (g/l)	26.9	25.1	1.5 (0.65)	0.02
ALP (IU/ml)	116.9	103.6	15.8 (4.48)	0.002

* abbreviations: SE, standard error of the mean; b.p., blood pressure; wbc, white blood count; ALP, alkaline phosphatase.

+ not statistically significant

TABLE 4 - POSSIBLE ADVERSE EFFECTS OF TREATMENT

Symptom	cyclosporin (n=32)	placebo (n=31)
Hypertrichosis	13	
Paraesthesiae	8	
Tremor	6	
Headache	6	2
Hypertension	4	
Influenza-like symptoms	4	1
Nausea, indigestion	2	2
Tiredness		2
Fluid retention	2	
Cramps	2	
Gingival hyperplasia	1	
Rash	1	
Herpes zoster	1	
Diverticulitis	1	1
Conjunctivitis		1
Facial swelling	1	
Facial flushing	1	
Classical migraine	1	
Total no. of adverse events	54	9
No. of patients reporting adverse events	24	8

EXAMPLE 2

CYCLOSPORIN A: USE IN BRONCHIECTASIS AND SINUSITIS

An experimental model of bronchiectasis was set up in the apical lobe of the rat by partial ligation of the apical lobe bronchus and distal intra-bronchial injection of viable bacteria. This model allowed for testing the pathogenetic mechanisms underlying bronchiectasis and for testing efficacy of treatment. The immunohistology in the experimental model closely resembled that of the human bronchiectasis, particularly with respect to evolution of a florid intra-mural cell-mediated immune response.

Results

(1) Experimental

In the experimental model of bronchiectasis it was shown that administration of cyclosporin A by the oral route (by gavage) from the day after surgery results in prevention of the evolution of bronchiectasis and of the intra-mural cell-mediated immune response and neutrophil traffic to the bronchial lumen. The relevant control for the vehicle (carrying the cyclosporin A) alone showed evolution of bronchiectasis, implying that cyclosporin A itself resulted in this prevention.

(2) Human

To date nine patients with proven bronchiectasis of very severe and idiopathic nature, who had not been improved by conventional medical treatment and who were

fully informed of the nature, rationale and possible adverse effects of the treatment, were treated by oral administration of cyclosporin A in open manner for periods ranging between 3 and 15 months. These patients had "end stage" bronchiectasis and efforts were either being made to keep them alive prior to lung transplantation, or to improve their quality of life in cases where lung transplantation was not a feasible option.

The rationale for treatment was that the drug could reduce the T lymphocyte intra-mural bronchial infiltration, as seen in the cyclosporin A-treated experimental animals, and possibly also reduce cytokine-driven recruitment of neutrophils to the bronchial lumen. Blood levels of cyclosporin were monitored regularly, as was renal function and blood pressure.

Seven out of nine of these patients demonstrated either reduction in sputum purulence and volume and improvement in respiratory function, or stabilisation of these parameters (which had been deteriorating in all patients prior to cyclosporin A treatment). In five improved cases in whom treatment was stopped after 3 months, deterioration occurred within eight weeks.

One patient with severe end-stage bronchiectasis of both lungs was treated with single lung transplantation and was administered cyclosporin A post-operatively as is the routine. In this patient the bronchiectasis in the remaining lung appeared to have become quiescent and

expectoration of sputum completely stopped, suggesting a beneficial effect of the drug in treating the residual bronchiectasis.

Conclusions

Cyclosporin A appears to be efficacious in the treatment of idiopathic bronchiectasis, even when this is severe, end-stage and threatening life.

EXAMPLE 3

IN VITRO TEST:

THE EFFECT OF DEXAMETHASONE AND CYCLOSPORIN A ON T LYMPHOCYTE PROLIFERATION IN VITRO

The present study compared the ability of dexamethasone (Dex) and cyclosporin A (CsA) to inhibit proliferation of T lymphocytes from clinically corticosteroid-resistant and -sensitive asthmatic patients.

Patient selection

Eleven patients with moderate to severe asthma (FEV_1 <70% of the predicted value) were characterised clinically as sensitive or resistant to oral prednisolone therapy according to their FEV_1 response to oral prednisolone 20mg/day for 7 days followed if necessary by 40mg/day for a further 7 days. Patients showing an increase in $FEV_1 \geq 15\%$ from baseline were classified as sensitive; the remainder were classified as resistant. Six sensitive and five resistant patients were recruited for the present study.

In selecting patients, strict criteria were invoked for the diagnosis of asthma and to rule out patients with irreversible airways obstruction. Thus, all patients were required to demonstrate reversibility of at least 20% in their PEFR or FEV_1 after inhaled β_2-agonist, and smokers were excluded.

Materials

Aliquots of drug stock solutions were filtered sterile through a 0.22μm pore size filter and stored at -20°C or as otherwise stated. Appropriate dilutions (as detailed below) were made up in RPMI-1640 for individual experiments. The same batch of stock solution was used for all experiments.

PHA (PHA-P, L9017, Sigma, Poole, England) was prepared at a concentration of 100μg/ml in RPMI-1640 and filtered sterile. This was stored in aliquots at -20°C, and was added to cultured cells at a final concentration of 5μg/ml.

Disodium dexamethasone was dissolved directly in RPMI-1640 to produce a concentration of 10^{-4} M.

Cyclosporin A: the oily suspension (100mg/ml) was diluted in ethanol, then in RPMI-1640 to produce a final concentration of 10^{-4} M.

In each experiment therefore the following drug concentrations were used:

Dexamethasone 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9} M

Cyclosporin A 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} M

Peripheral blood mononuclear cell (PBMC) isolation and culture

50 ml of peripheral blood was taken into preservative-free heparin in a sterile fashion. Blood was mixed with an equal volume of RPMI-1640 medium (Gibco, Paisley, Scotland). Thirty ml aliquots were layered onto 20ml aliquots of Ficoll-Paque (Pharmacia, Uppsala, Sweden) in 50ml sterile conical tubes ("Falcon", Becton Dickinson, Cowley, England). After centrifuging at 400g for 20 min at 20°C, PBMC were removed from the plasma/Ficoll interface using gentle suction, transferred to sterile polystyrene universal containers and washed twice with RPMI-1640.

PBMC isolated as described above were resuspended in RPMI-1640 at 4 x 10^6/ml. 80µl aliquots of this cell suspension with or without added PHA (5µg) were added to 80µl aliquots of drug solution or medium control in triplicate in sterile 96 well round bottomed culture plates (Cel-cult, Sterilin).

Culture plates were incubated for 48 hours in a humidified atmosphere with 5% CO_2. Cell proliferation was measured by uptake of tritiated methylthymidine. Sterile tritiated thymidine solution (Amersham) (0.66µCi/well in a volume of 10µl) was added to cell culture wells for the last 6-8 hours of the 48 hour incubation period. After incubation, cells were harvested onto glass-fibre filter paper using a cell

harvester apparatus (Skatron Instruments Ltd) and the incorporated radio-label counted using a beta-spectrometer. Results were expressed as mean counts of triplicate cultures and dose-response curves constructed for each drug for percentage inhibition of the positive (no drug) control.

Measurement of the effects of Dex and CsA on the proliferative response of T lymphocytes

PBMC were resuspended in RPMI-1640 at 4 x 10^6/ml. 80µl aliquots of this cell suspension with or without added PHA were added to 20µl aliquots of drug solution or medium control in triplicate in sterile 96 well U-bottomed culture plates as described above. 60µl of medium were added to give a total volume of 160µl and final concentrations of the cell suspension ($2x10^6$ cell/ml); PHA (5µg/ml); and the drug solutions listed above. Culture plates were incubated for 48 hours and the proliferation was measured as described above.

Statistical methods

All results are expressed as Mean ± standard error of the mean (SEM). Significance levels were calculated using Mann-Whitney test.

Results

Addition of both drugs to peripheral blood mono-nuclear cell suspensions at a concentration range of 0.1 nM to 1000 nM resulted in a dose-dependent

suppression of PHA-induced proliferation.

Ethanol and CsA vehicle dilution control experiments showed no effect on lymphocyte proliferation at the concentrations used in the lowest drug dilutions.

PBMC from the clinically corticosteroid-resistant asthmatic patients were significantly less sensitive to Dex than those of sensitive patients ($p<0.01$ at $10^{-6}M$; $p<0.05$ at $10^{-7}M$ and at $10^{-9}M$ concentration levels) (Fig. 2). Cyclosporin A inhibited cells from both patient groups to an equivalent extent.

Conclusions

T-lymphocytes from clinically corticosteroid-resistant asthmatic patients were significantly more resistant to the inhibitory action of dexamethasone in vitro as compared to those of corticosteroid-sensitive patients. CsA inhibited lymphocyte proliferation in both groups of patients.

PATHOLOGICAL EXAMPLE 1

IMMUNOHISTOLOGICAL STUDY OF BRONCHIAL MUCOSA IN ASTHMA

Fibre-optic bronchial biopsies were obtained from 5 intrinsic asthmatics (I), 9 occupational (5 toluene and 4 methylene diisocyanate sensitive) asthmatics (O) not exposed in the previous 4 weeks, 6 extrinsic asthmatics (E) and 12 normal control subjects (N). We examined the phenotype and activation status of leukocytes in the bronchial submucosa by standard immunohistology

techniques using a panel of monoclonal antibodies. The results for the asthmatic groups were compared with normal controls. The median cell counts/unit length of basement membrane were as follows:

TABLE 5

	I	O	E	N
CD45	113*	57	74	58
CD3	87*	35	39	37
CD4	51*	19	21	19
CD8	1	6	0.3	0
CD25(IL-2R)$^+$	2**	0.5**	0.2	0
EG2	13*	5***	17***	0

* $p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$

$^+$ IL-2R = interleukin 2 receptor

No significant differences in neutrophil counts (neutrophil elastase positive cells) were observed in any asthmatic group compared with normal controls. These results identify comparable findings in occupational and extrinsic asthma, more pronounced in intrinsic asthma, and support the hypothesis that T-lymphocyte/eosinophil interactions are important in the pathogenesis of asthma of diverse aetiology.

PATHOLOGICAL EXAMPLE 2

CYTOKINE mRNA IN BRONCHOALVEOLAR LAVAGE IN ATOPIC ASTHMA

Methods. Bronchoalveolar lavage cells from 10 mild atopic asthmatics (methacholine PC_{20} 0.25-16.0 mg/ml) and

10 normal control subjects (PC_{20} > 32mg/ml) were assessed for expression of mRNA for IL-2, IL-3, IL-4, IL-5, GM-CSF, and interferon-gamma (IFN-gamma) by in situ hybridization using ^{32}P-labelled riboprobes. Localisation of mRNA to bronchoalveolar lavage (BAL) T-cells was assessed by simultaneous in situ hybridization and immunofluorescence and by in situ hybridization after immunomagnetic enrichment or depletion of T-cells.

Results. As shown in Table 6 below, there was increased expression of mRNA for cytokines in asthmatics:

TABLE 6

mRNA+ cells per 1000

Median (95% confidence intervals)

	Asthmatics (n = 10)	Controls (n = 10)	p
IL-2	13.5 (0-22)	4.5 (0-10)	p<0.05
IL-3	18.5 (12-30)	5.5 (0-11)	<0.01
IL-4	28.0 (20-36)	4.0 (0-8)	<0.001
IL-5	28.0 (18-40)	6.5 (0-13)	<0.001
GM-CSF	38.0 (27-55)	8.5 (0-15)	<0.001
IFN-gamma	2.5 (0-19)	0 (0-10)	ns

Compared to control subjects, asthmatics had significantly higher numbers of bronchoalveolar cells per 1000 positive for mRNA for IL-2, IL-3, IL-4, IL-5 and GM-CSF. There was no difference between the two groups in numbers of cells expressing mRNA for IFN-gamma. In asthmatic subjects mRNA for IL-4 and IL-5 was

predominantly expressed by T lymphocytes. There was increased expression of CD25 (IL-2R) on CD4+ BAL lymphocytes in asthmatics (median 13.6% vs 8.7%, $p < 0.001$) and an increased percentage of eosinophils in BAL (4.0% vm 0.4%, $p < 0.001$). Asthmatics also showed significant correlations between IL-5 mRNA+ cells and %CD25+CD4+ T cells ($r = 0.822$, $p < 0.02$), and %CD25+CD4+ lymphocytes and BAL eosinophils ($r = 0.733$, $p < 0.05$). For all subjects IL-5 mRNA+ cells correlated with BAL eosinophils ($r = 0.706$, $p < 0.001$).

Conclusion. Atopic asthma is associated with activation of the IL-3, IL-4, IL-5 and GMCSF gene cluster. This may be due to predominant activation of a T_{H2} equivalent T-cell population.

PATHOLOGICAL EXAMPLE 3

T LYMPHOCYTES IN AIRWAY MUCOSA IN FATAL ASTHMA AND CYSTIC FIBROSIS

Using specific monoclonal antibodies (mAb) and immunohistology, the numbers of phenotypically distinct cells infiltrating lung tissue from 15 post-mortem (PM) cases of fatal asthma were quantified and compared with 6 cases of cystic fibrosis (CF) (3 post-mortem, 3 transplant) and 10 non-asthmatic cases of sudden death matched for age and sex.

Tissue fixation

Blocks of lung tissue were or had been fixed in neutral buffered formalin for several days, processed in graded alcohols by standard methods and embedded in paraffin wax. Three to five micron paraffin sections of formalin-fixed segmental and subsegmental airways were cut using a sledge or rotary microtome, and retrieved on albumin-coated slides, dried at 60°C for 1 hour, and then stored until stained.

Immunohistology

The mouse monoclonal antibody (mAb) MT1 (Bionuclear Services, Reading, Berks, UK) - 1/5 dilution) was used for the examination of the paraffin-embedded tissue. This stains the 110-160 kD membrane proteins associated with the CD43 antigen, sialophorin (ie staining T cells with little B cell or macrophage specificity).

MAb staining was detected using a modification of the immunogold silver staining (IGSS) technique, originally described by Holgate et al.

Quantification

All tissue sections were coded and counted in a blinded fashion. Counts were made of intrapulmonary bronchi of diameter range 0.5-1.5cm (measured as the minimum diameter between the epithelial basement membranes). The number of each positively-stained cell was counted in a zone 115μm deep to the epithelial basement membrane (defined by a squared eyepiece graticule) along

the entire length of the basement membrane. Counts were also made within the epithelium. A calibrated graphics tablet, conected to an Apple IIe computer, was used to determine the length of the epithelial basement membrane along which sub-epithelial tissue was assessed. All counts were expressed/unit length (1000μm) basement membrane (BM).

Results

The mean results (±SEM) of cell counts following application of each antibody are shown in Figure 3.

This figure shows the number of immuno-positive leukocytes (MT-1 positive (T) cells) detected by the IGSS technique expressed per mm length epithelial basement membrane in a zone 115μm deep to the lamina reticularis in the asthmatic (A), cystic fibrosis (CF) and control (C) groups. Horizontal bars denote the median values.

Lymphocyte infiltration of the airway mucosa by MT-1 positive (T) lymphocytes was found in all three groups, being significantly higher than the controls in both the asthma and CF groups. The asthma and CF groups had mean and median values for the group more than twice that of the non-asthma sudden death group ($p<0.05$ and $p<0.01$ respectively).

The results support a role for the T lymphocyte in the pathogenesis of fatal asthma and CF.

CLAIMS

1. Use of cyclosporin A or other immunosuppresive agent with a common mode or site of action, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

2. Use of a T cell-selective immunosuppressive agent for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

3. Use of an immunosuppressive agent that inhibits cytokine elaboration, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis.

4. Use as claimed in any one of claims 1 to 3, wherein the immunosuppressive agent is one that is selective for the IL-4 family of cytokines.

5. Use as claimed in any one of claims 1 to 4, wherein the immunosuppressive agent is FK506.

6. Use as claimed in any one of claims 1 to 4, wherein the immunosuppressive agent is rapamycin.

7. Use as claimed in any one of claims 1 to 4, wherein the immunosuppressive agent is a humanised anti-CD4 antibody.

8. Use as claimed in any one of claims 1 to 4, wherein the immunosuppressive agent is a humanised antibody directed against the IL-2 receptor and/or

against one or more of IL-5, IL-4, IL-3, GM-CSF, IL-6, IL-8 and TNF.

9. Use as claimed in any one of claims 1 to 8, wherein the medicament also contains misoprostol.

10. Use as claimed in any one of claims 1 to 9, wherein the medicament is in a form suitable for administration by inhalation.

11. A method for the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, wherein there is administered a therapeutically effective dose of cyclosporin A or other immunosuppressive agent with a common mode or site of action.

12. A method for the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, wherein there is administered a therapeutically effective dose of a T cell-selective immunosuppressive agent.

13. A method for the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, wherein there is administered a therapeutically effective dose of an immunosuppressive agent that inhibits cytokine elaboration.

14. A method as claimed in any one of claims 11 to 13, wherein the immunosuppressive agent is one that is selective for the IL-4 family of cytokines.

15. A method as claimed in any one of claims 11 to 14, wherein FK506 is used.

16. A method as claimed in any one of claims 11 to 14, wherein rapamycin is used.

17. A method as claimed in any one of claims 11 to 14, wherein a humanised anti-CD4 antibody is used.

18. A method as claimed in any one of claims 11 to 14, wherein a humanised antibody directed against the IL-2 receptor and/or against one or more of IL-5, IL-4, IL-3, GM-CSF, IL-6, IL-8 and TNF is used.

19. A method as claimed in any one of claims 11 to 18, wherein misoprostol is also used.

20. A method as claimed in any one of claims 11 to 14 and 19, wherein cyclosporin A is used in an amount of no more than 5 mg of active ingredient per kg of body weight per day.

21. A method as claimed in any one of claims 11 to 20, wherein administration is by inhalation.

22. A method as claimed in any one of claims 11 to 21, for the treatment of severe chronic asthma.

23. A method as claimed in any one of claims 11 to 21, for the treatment of mild or moderate asthma.

24. A method as claimed in any one of claims 11 to 21, for the treatment of bronchiectasis or cystic fibrosis.

25. A pharmaceutical preparation for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises cyclosporin A or other immunosuppressive agent with a common mode or site

of action and being suitable for administration by inhalation, in admixture or conjunction with a pharmaceutically suitable carrier, and which is in a form suitable for inhalation.

26. A pharmaceutical preparation for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises a T cell-selective immunosuppressive agent suitable for administration by inhalation, in admixture or conjunction with a pharmaceutically suitable carrier, and which is in a form suitable for inhalation.

27. A pharmaceutical preparation for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises an immunosuppressive agent that inhibits cytokine elaboration and that is suitable for administration by inhalation, in admixture or conjunction with a pharmaceutically suitable carrier, and which is in a form suitable for inhalation.

28. A pharmaceutical preparation as claimed in any one of claims 25 to 27, wherein the immunosuppressive agent is one that is selective for the IL-4 family of cytokines.

29. A method of predicting clinical response to a corticosteroid or an immunosuppressive agent in a treatment for a disease characterised by airflow obstruction and/or of chronic sinusitis, which comprises ascertaining the effect of a corticosteroid or

immunosuppressive agent on the response of T-lymphocytes or T-lymphocyte blasts *in vitro* to treatment with a stimulant or mitogen, an inhibitory effect by the corticosteroid or immunosuppressive agent on the response of the T-lymphocytes or T-lymphocyte blasts to the stimulant or mitogen being indicative of a positive response *in vivo* to therapy by a corticosteroid or immunosuppressive agent respectively and/or an absence of such inhibitory effect being indicative of a negative response *in vivo* to therapy by a corticosteroid or immunosuppressive agent respectively.

30. A method as claimed in claim 29, wherein the test compound used *in vitro* in the T-lymphocyte or T-lymphocyte blast treatment is the same as that for which prediction of response of the patient to therapy is sought.

31. An *in vitro* test method of predicting the response *in vivo* of a patient to a method of treatment as specified in any one of claims 11 to 24, which comprises ascertaining the effect of an immunosuppressive agent as specified in any one of claims 11 to 18 on the response of T-lymphocytes or T-lymphocyte blasts obtained from the patient to treatment with a stimulant or mitogen, an inhibitory effect by the immunosuppressive agent on the response of the T-lymphocytes or lymphocyte blasts to the stimulant or mitogen being indicative of a positive response *in vivo* of the patient to therapy using an

immunosuppressive agent as specified in any one of claims 11 to 18.

32. A method as claimed in any one of claims 29 to 31, wherein the effect of a combination of misoprostol and the immunosuppressive agent is ascertained.

33. A method of screening an immunosuppressive agent for use in the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, which comprises ascertaining the effect of the immunosuppressive agent on the response of T-lymphocytes or T-lymphocyte blasts in vitro to treatment with a stimulant or mitogen, an inhibitory effect by the immunosuppressive agent on the response of the T-lymphocytes or T-lymphocyte blasts to the stimulant or mitogen being indicative of a positive response in vivo to therapy by the immunosuppressive agent.

34. A method as claimed in any one of claims 29 to 33, wherein the response of the T-lymphocytes is assessed by measurement of uptake of tritiated thymidine by the T-lymphocytes and/or by measurement of one or more cytokines produced by the T-lymphocytes.

35. A method as claimed in any one of claims 29 to 34, wherein the mitogen is phytohaemagglutinin.

36. A method for the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, which comprises carrying out an in vitro test as specified in any one of claims 29 to 35 on T-lympho-

cytes or T-lymphocyte blasts obtained from the patient, and administering a therapeutically effective amount of a compound that in the in vitro test was indicative of a positive response in vivo.

37. A method for the treatment of a disease characterised by airflow obstruction and/or of chronic sinusitis, wherein there is administered a therapeutically effective amount of an immunosuppressive agent that inhibits the response of T-lymphocytes or T-lymphocyte blasts in vitro to treatment with a stimulant or mitogen.

38. A method of treatment as claimed in any one of claims 11 to 24, which is carried out on a patient from whom the T-lymphocytes or T-lymphocyte blasts have been tested by a method as specified in any one of claims 29 to 35 which was indicative of a positive response in vivo.

39. A method of treatment as claimed in any one of claims 11 to 24, which is carried out on a patient from whom the T-lymphocytes or T-lymphocyte blasts have been tested using a corticosteroid by a method as specified in claim 29 which test was indicative of a negative response in vivo to corticosteroid treatment.

40. Use of an immunosuppressive agent that in an in vitro test as specified in any one of claims 29 to 35 is indicative of a positive response in vivo, for the manufacture of a medicament for the treatment of diseases characterised by airflow obstruction and/or of chronic

sinusitis.

41. Use as claimed in any one of claims 1 to 10, wherein the immunosuppressive agent is one that inhibits the response of T-lymphocytes or T-lymphocyte blasts *in vitro* to treatment with a stimulant or mitogen.

42. A pharmaceutical preparation for the treatment of diseases characterised by airflow obstruction and/or of chronic sinusitis, which comprises an immunosuppressive agent suitable for administration by inhalation and that in an *in vitro* test as specified in any one of claims 29 to 35 is indicative of a positive response *in vivo*, in admixture or conjunction with a pharmaceutically suitable carrier, and which is in a form suitable for inhalation.

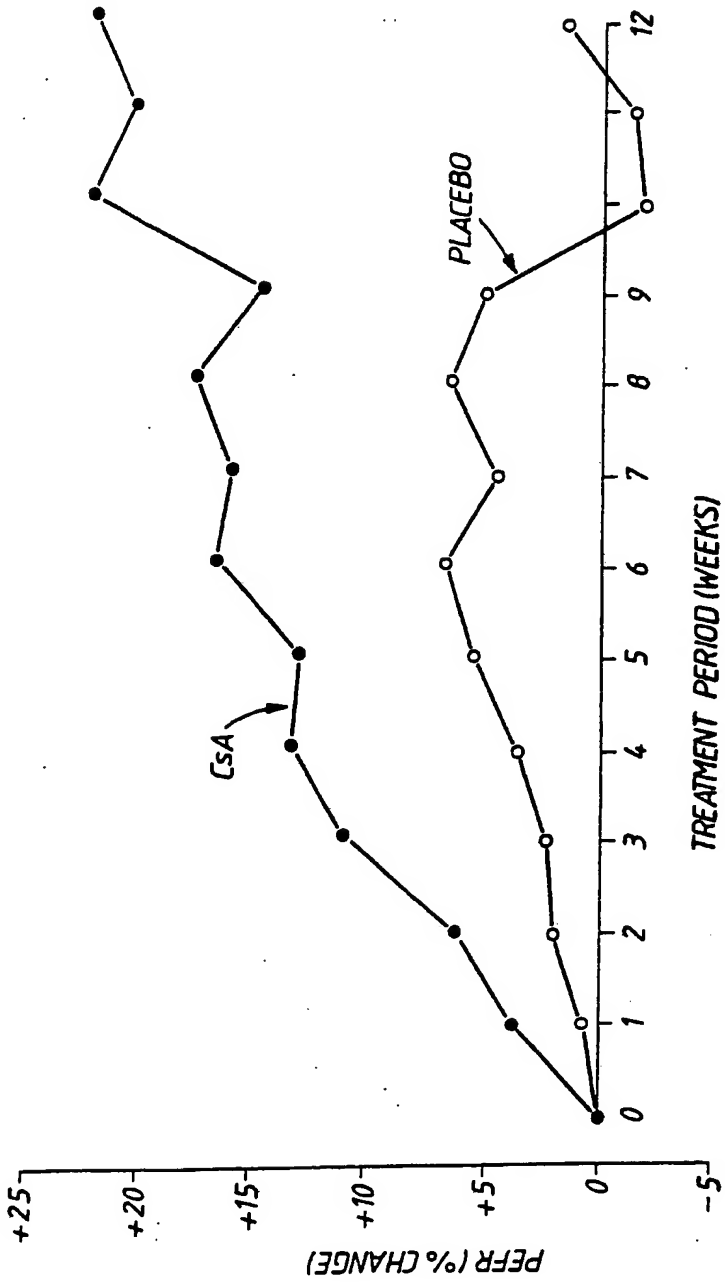
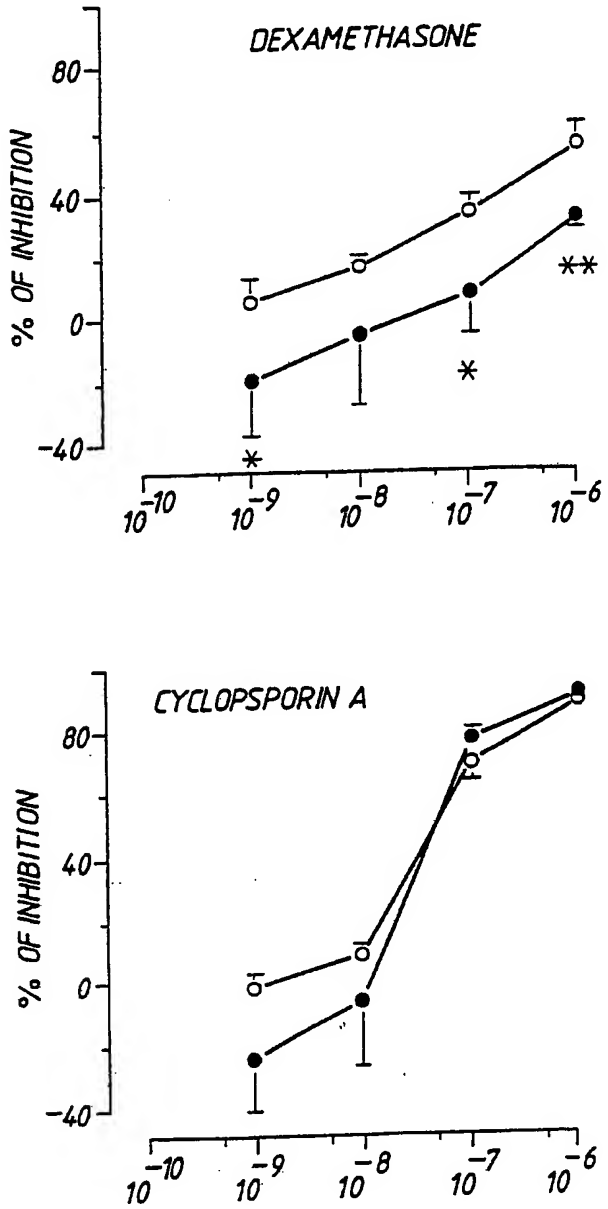

PEFR (% CHANGE)
+25
+20
+15
+10
+5
0
-5
TREATMENT PERIOD (WEEKS)
0
1
2
3
4
5
6
7
8
9
12
CsA
PLACEBO

Fig.1.



PERCENTAGE INHIBITION OF PROLIFERATION AS FUNCTION OF DRUG CONCENTRATION

● CLINICALLY RESISTANT PATIENTS N=5;

○ CLINICALLY SENSITIVE PATIENTS N=6

* P<0.05; ** P<0.01;

Fig.2.

IMMUNO-POSITIVE CELLS IN BRONCHI POST-MORTEM:
ASTHMA (A), CYSTIC FIBROSIS (CF), AND CONTROLS (C).

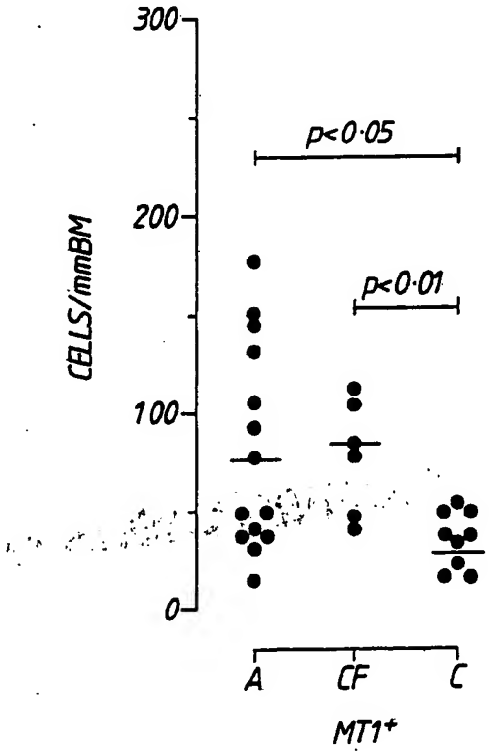


Fig.3.